Original Article

Correlation of serum trace elements (Fe, Cu, and Zn) in the blood samples of Indian patients with leukoplakia, oral squamous cell carcinoma, and normal subjects

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Abstract

Context: Trace elements (iron, copper, and zinc), leukoplakia, and oral squamous cell carcinoma.

Aims: To study and correlate serum trace element (iron, copper, and zinc) levels in leukoplakia, oral squamous cell carcinoma, and normal subjects.

Settings and Design: The present study comprised a total of 80 patients, which included 30 patients of leukoplakia, 30 patients of oral squamous cell carcinoma, and the normal control group comprising 20 healthy individuals who were not having any relevant medical, dental, and habit history.

Subjects and Methods: Peripheral blood samples of a volume of 10 ml each will be collected by anti-cubical vein puncture from control groups and from patients suffering from leukoplakia and oral squamous cell carcinoma. The blood will be collected in a plain red top vein puncture tube without additives or anti-coagulants and allowed to stand undisturbed to clot at room temperature, and serum will be separated from the cells by centrifugation at 4°C at a speed of 3000 rev/min, the separated sera will be kept at –20°C until analysis is performed.

Estimation of Serum Iron, Zinc, and Copper Levels: The estimation of serum zinc (Zn) and copper (Cu) levels is performed by atomic absorption spectrometry (AAS). In the present study, the estimation of copper and zinc levels was conducted by using an atomic absorption spectrophotometer (model no: AA-6300 SHIMADZU, Japan). Serum iron estimation is performed using the kit RANDOX (siedel, 1984).

Statistical Analysis Used: Statistical analysis is performed by the paired and Scheffe tests.

Results: From the results, it was concluded that there was a decrease in serum iron and zinc levels and copper had increased serum levels.

Conclusions: It was concluded that evaluation of serum trace elements can be a cost-effective and non-invasive alternative for screening, diagnosis, and monitoring of pre-malignant lesions such as leukoplakia and malignant lesions such as oral squamous cell carcinoma. Thus, these parameters can be

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Submitted: 21-Jan-2022, **Revised:** 15-Feb-2022, **Accepted:** 22-Mar-2022, **Published:** 21-Mar-2023

Access this article online

Quick Response Code:

Website: www.jomfp.in

DOI: 10.4103/jomfp.jomfp_29_22

How to cite this article: Sourabh A, Shreedhar B, Khare A, Haideri S, Kalim S, Srivastava A. Correlation of serum trace elements (Fe, Cu, and Zn) in the blood samples of Indian patients with leukoplakia, oral squamous cell carcinoma, and normal subjects. J Oral Maxillofac Pathol 2023;27:76-9.

used as biomarkers that provide important tools in formulating an adequate diagnosis, treatment plan, and prognosis for oral squamous cell carcinoma.

Keywords: Leukoplakia, oral squamous cell carcinoma, serum, trace elements

INTRODUCTION

An element which is required in amounts smaller than 0.01% of the mass of the organism is called a trace element. However, all essential trace metals become toxic when their concentration becomes excessive.[1]

The most common neoplasm is supposed to be the cancer of oral cavity in developing countries.

Leukoplakia is most common oral potentially malignant lesion, with a higher tendency of malignant transformation increased with follow-up years.

Recently, trace elements have received much attention in the detection of oral cancer and pre-cancer as they are found significantly altered in the head and neck, lung, and breast carcinomas.[2] The ratio of copper to zinc is also found as a dependable biomarker in the development and progression of carcinogenesis.

Hence, the present study was undertaken keeping this fundamental importance in mind. Here, we have assessed and compared the levels of serum trace elements in normal individuals, oral leukoplakia patients, and oral squamous cell carcinoma patients in a bid to arrive at a parameter which will help us in early diagnosis and a better treatment plan and thus a better prognosis.

SUBJECTS AND METHODS

The present study was conducted within the Department of Oral Pathology and Microbiology in Career Post Graduate Institute of Dental Sciences and Hospital, Lucknow. The study participant included 20 normal healthy volunteers, 30 patients with diagnosis of leukoplakia, and 30 patients with the diagnosis of oral squamous cell carcinoma.

Inclusion criteria

The age was 25–60 years irrespective of gender. In Group I, healthy volunteers having no oral lesion and not having the habit of tobacco or alcohol use were included; in Group II, patients with the confirmed diagnosis of leukoplakia were included; and in Group III, patients with the confirmed diagnosis of oral epithelial cell carcinoma were included within the study.

Exclusion criteria

Cases with a known history of systemic disorder were excluded. Also, immunocompromised patients and patients affected by mental disease were excluded, and treated cases of leukoplakia and oral epithelial cell carcinoma were excluded from the study. Subjects who are taking any trace element supplements were also excluded.

Sample collection

The participants were explained intimately about the procedure, and a signed consent form was taken from them.

METHOD

Blood samples were collected from each subject after overnight fasting. Five millilitres of blood from antecubital veins was drawn from the selected patients using a sterile disposable syringe taking full precautions to prevent haemolysis. Five millilitres of the blood sample was kept into the plain red top vein puncture tube without additives or anti-coagulants; the sample was then allowed to clot at room temperature for about 2 hours and was then centrifuged at 3000 rpm for 10 minutes to separate the serum. Immediately, the serum was kept at -20°C for the analysis of trace elements.

ESTIMATION OF TRACE ELEMENTS (IRON, COPPER, AND ZINC) BY INDUCTIVELY COUPLED PLASMA–OPTICAL EMISSION SPECTROMETRY (ICP–OES) ANALYSIS

Experimental procedure

A volume of 0.5 ml of sera is taken from the already stored samples, the sample is allowed to attain room temperature, and then the sample is diluted with 3 ml of milli-Q water. The diluted sample is then kept in the collecting tube of the spectrophotometer, and the result is obtained on the linked computer through the software system.

The resulting clear solution after Molecular dynamics (MD) is analyzed by ICP–OES.

Table 1: Basic characteristics of three groups

Basic characteristics	Normal (*n*=20) (%)	Leukoplakia (*n*=30) (%)	Oral squamous cell carcinoma (*n*=30) (%)	F/χ^2	*P*
Age (years): Mean±SE	48.90±2.84	46.97±2.56	49.70±2.60	0.31	0.737
Sex:	5 (25.0)	3 (10.0)	2 (6.7)	3.96	0.138
Female	15 (75.0)	27 (90.0)	28 (93.3)		
Male					

Statistical analysis

Data were summarised as mean ± SE (standard error of the mean). Groups were compared by one-way analysis of variance (ANOVA), and the significance of the mean difference between (inter) the groups was performed by Tukey's HSD (honestly significant difference) test after ascertaining normality by Shapiro–Wilk's test and homogeneity of variance by Levene's test. Discrete (categorical) groups were compared by Chi-square (χ^2) test. A two-tailed ($\alpha = 2$) *P* value less than 0.05 ($p < 0.05$) was considered statistically significant. Analyses were performed on SPSS software (windows version 17.0).

Table 2: Levels of trace elements in three groups

Group	*n*	Mean±SE	*F*	*P*
Iron levels in three groups				
Normal	20	186.45±6.87	7.22	0.001
Leukoplakia	30	162.83±5.09		
Oral squamous cell carcinoma	30	160.46±3.25		
Copper levels in three groups				
Normal	20	136.01±22.70	0.40	0.669
Leukoplakia	30	176±26.62		
Oral squamous cell carcinoma	30	163.38±35.35		
Zinc levels in three groups				
Normal	20	38.29±2.74	4.17	0.019
Leukoplakia	30	28.49±3.80		
Oral squamous cell carcinoma	30	25.46±2.02		

DISCUSSION

In our result, Table 1 shows the sample size distribution considering age and sex. Age shows a non-significant *P* value of 0.737, and sex shows a non-significant *P* value of 0.138. Thus, it is indicative of the fact that all the patients that we have included in our study are of almost the same age group, whereas distribution among sex shows that all groups have almost an equivalent number of female and male patients.

In our result, Table 2 shows a significant *P* value of 0.001, showing that a significant difference was observed between normal, leukoplakia patients and oral squamous cell carcinoma patients, whereas non-significant values of copper levels were seen among normal, leukoplakia, and oral squamous cell carcinoma individuals. A moderately significant correlation of zinc level was seen among three groups of patients.

Similarly for copper, Table 2 shows serum copper levels in which the *P* value is non-significant and the copper levels are maximum in leukoplakia, followed by oral squamous cell carcinoma, and last in the normal group.

Table 3 shows the inter-group comparison and difference in copper levels for which the *P* value is non-significant and the mean difference of copper levels is maximum for normal versus leukoplakia, followed by normal versus oral squamous cell carcinoma and last leukoplakia versus oral squamous cell carcinoma. The findings of copper levels were in accordance with the previous studies performed by Singh *et al*., Balpande *et al*., Shetty *et al*., and Arvind rao, Kumar *et al*., and Krishna *et al*.;[3] the reason for increased levels of serum copper in leukoplakia patients could be correlated with other studies, which shows that areca nuts have a high copper content and it is one of the major etiological agents in the pre-cancerous group of patients such as oral sub-mucous fibrosis and leukoplakia.[2,4-7]

Table 3 shows the inter-group comparation between *P* values of normal individuals, leukoplakia patients, and oral squamous cell carcinoma patients. The significant values of iron level are getting depleted in leukoplakia and oral squamous cell carcinoma. A non-significant *P* value was seen in copper and zinc.

The statistically significant reduction in serum iron level in normal versus leukoplakia and normal versus oral squamous cell carcinoma could be because of utilisation of iron by the bone marrow and tumours as reported by Chellacombe *et al*.[8] Khanna *et al*.[9] stated that there appears to be an association between the serum iron content and oral carcinogenesis. A more detailed study on a large database could be instituted to elucidate the exact role of iron.[10]

There is a non-significant *P* value on comparison of the copper level between the normal versus leukoplakia, normal versus oral squamous cell carcinoma, and leukoplakia versus oral squamous cell carcinoma groups and the zinc level between the normal versus leukoplakia, normal versus oral squamous cell carcinoma, and leukoplakia versus oral squamous cell carcinoma groups. The reason for this could be attributed to the inter-correlation between copper and zinc. Balpande *et al*. also similarly stated that there is a negative interaction between copper and zinc and an increase in copper level may cause subsequent reduction in zinc level as well.[8]

Table 3: Comparison (P) of the mean difference in Fe,Cu, Zn level between the groups by Tukey test

Comparison	Mean difference	*q*	*P*	95% CI of difference
Comparison (*P*) of the mean difference in Fe level between the groups by Tukey test				
Normal vs. Leukoplakia	23.61	4.56	0.005	6.06-41.16
Normal vs. Oral squamous cell carcinoma	25.99	5.02	0.002	8.44-43.54
Leukoplakia vs. Oral squamous cell carcinoma	2.377	0.51	0.930	-13.32-18.08
Comparison (*P*) of the mean difference in Cu level between the groups by Tukey test				
Normal vs. Leukoplakia	40.60	1.27	0.645	-149.20-67.98
Normal vs. Oral squamous cell carcinoma	27.37	0.85	0.819	-135.90-81.20
Leukoplakia vs. Oral squamous cell carcinoma	13.23	0.46	0.943	-83.89-110.30
Comparison (*P*) of the mean difference in Zn level between the groups by Tukey test				
Normal vs. Leukoplakia	9.80	3.06	0.085	-1.06-20.66
Normal vs. Oral squamous cell carcinoma	12.83	4.00	0.016	1.97-23.70
Leukoplakia vs. Oral squamous cell carcinoma	3.03	1.06	0.736	-6.68-12.75

CONCLUSION

Evaluation of serum trace elements can be a cost-effective and non-invasive alternative for screening, diagnosis, and monitoring of pre-malignant lesions such as leukoplakia and malignant lesions such as oral squamous cell carcinoma. Thus, these parameters can be used as biomarkers that provide important tools in formulating an adequate diagnosis, treatment plan, and prognosis for oral squamous cell carcinoma.

To reiterate our study, a further in depth research in this field is needed with a larger sample size for early diagnosis, management, and better prognosis of oral leukoplakia and oral squamous cell carcinoma.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

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